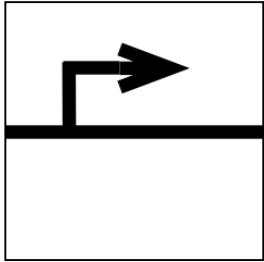
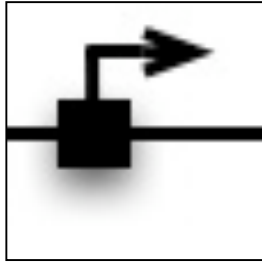


# Construction Plan Overview

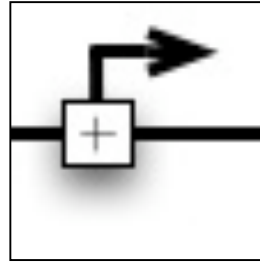
# Quick vBOL Key



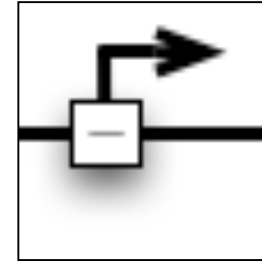
Forward Promoters



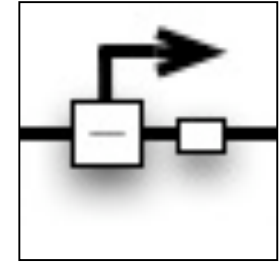
Constitutive



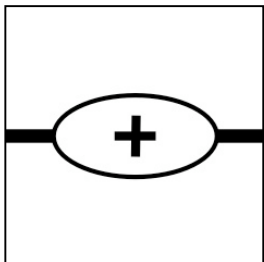
Inducible



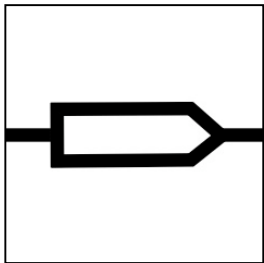
Repressible



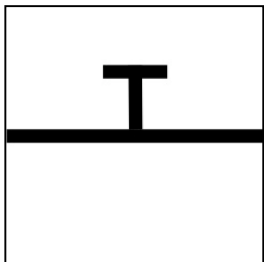
Promoter +  
Operator



Forward Ribosome Binding Sight



Open Reading Frame (gene)



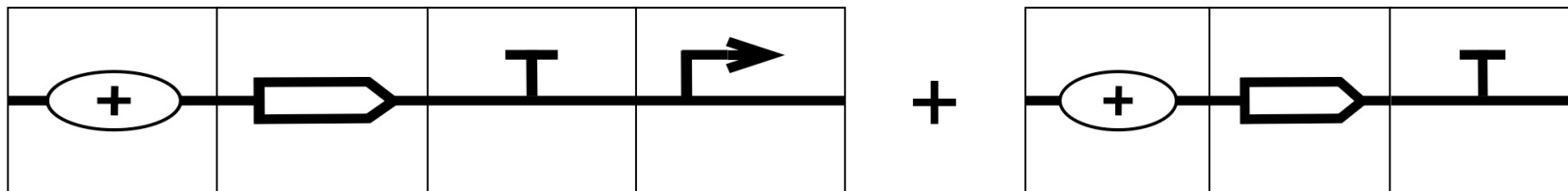
Terminator

# Device 1

## First Round of Cloning

### 1.1a SoxR/S Promoter + GFP tag

(Synthesized part # 54) + (BioBrick part # I13504)



B0034 + k223000 + B0015 + k2230001

(RBS) (SoxR gene) (TT) (SoxS Promoter)

Cut w/ EcoR1/Spe1

B0034 + E0040 + B0015

(RBS) (GFP) (TT)

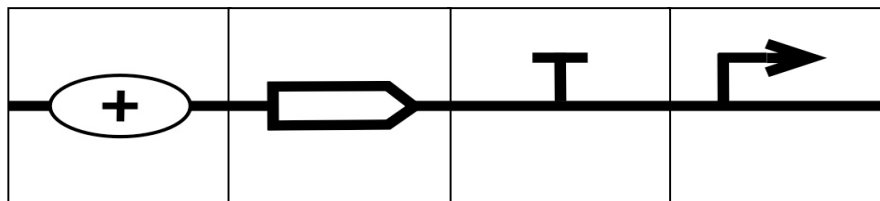
Cut w/ EcoR1/XbaI

# Device 1

## First Round of Cloning

1.1b SoxR/S Promoter +  
(Synthesized part # 54) +

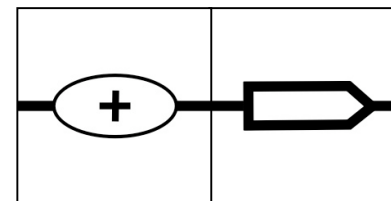
Blh parts  
(Synthesized part # 57)



B0034 + k223000 + B0015 + k2230001  
(RBS) (SoxR gene) (TT) (SoxS Promoter)

Cut w/ EcoR1/Spe1

+



B0034 + k223035  
(RBS) (Blh gene)

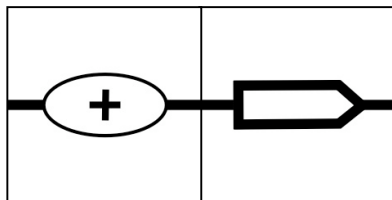
Cut w/ EcoR1/XbaI

# Device 1

## First Round of Cloning

1.1c      Blh gene                      +              Terminator

(Synthesized part # 57) + (BioBrick part # B0015)

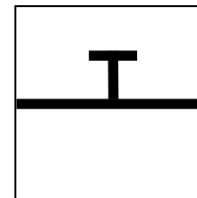


B0034 + k223035

(RBS)

(Blh gene)

+



B0015

(TT)

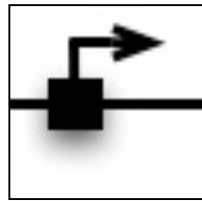
Cut w/ EcoR1/Spe1

Cut w/ EcoR1/XbaI

# Device 1

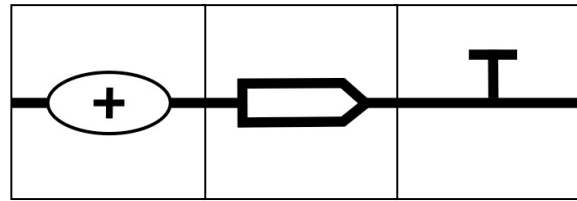
## First Round of Cloning

1.1d Promoter + Blh gene w/ Terminator  
(# J23100) + (Part from Cloning 1.1c)



J23100  
Promoter)

+



B0034 + k223035 + B0015  
(RBS) (Blh gene) + (TT)

Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

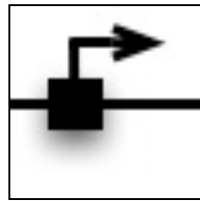
# Device 1

Keep!!!

## First Round of Cloning

1.1e Promoter + Crt Cluster (Beta-Carotene Producers)

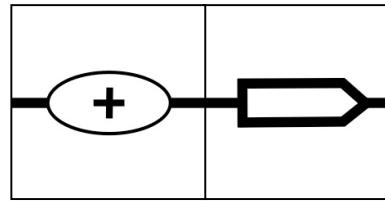
(# J23100) + (Synthesized Part on way )



J23100

Promoter)

+



B0034 + (?)

(RBS)

(crt genes = B,E, I, Y, and X)

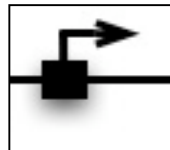
Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

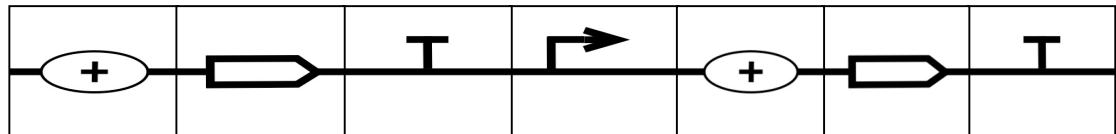
# Device 1

## Second Round of Cloning

1.2a Promoter + SoxR/S with GFP tag  
(J23100) + (Made From cloning step 1.1a)



+



J23100

Promoter)

B0034 + k223000 + B0015 + k2230001+ B0034 +E0040 + B0015

(RBS)

(SoxR gene)

(TT)

(SoxS Promoter)

(RBS)

(GFP)

(TT)

Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

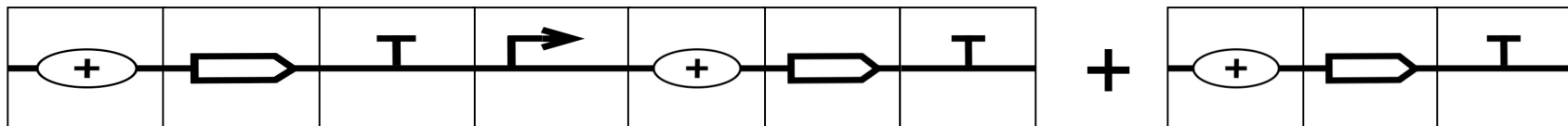


# Device 1

## Second Round of Cloning

1.2b SoxR/S w/ Blh + RALDH II group

(Cloned part from 1.1b) + (Synthesized part # 52)



B0034 + k223000 + B0015 + k2230001 + B0034 + k223035 + B0015  
(RBS) (SoxR gene) (TT) (SoxS Promoter) (RBS) (Blh gene) (TT)

B0034 + k223036 + B0015  
(RBS) (RALDH II gene) (TT)

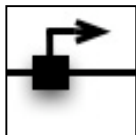
Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

# Device 1

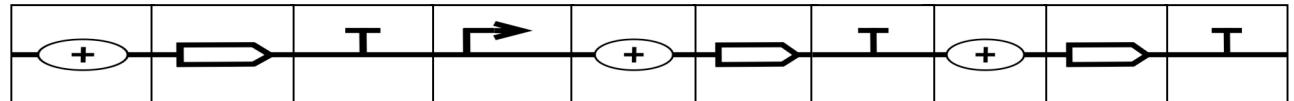
## Third Round of Cloning

1.3a Promoter + SoxR/S w/ Blh and RALDH II groups  
(Part #J23100) + (Cloned from step 1.2b)



J23100  
Promoter

+



B0034 + k223000 + B0015 + k2230001 + B0034 + k223035 + B0015 + B0034 + k223036 + B0015  
(RBS) (SoxR gene) (TT) (SoxS Promoter) (RBS) (Blh gene) (TT) (RBS) (RALDH II gene) (TT)

Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1



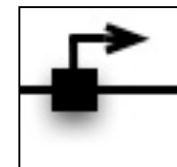
# Device 1 Controls

Testing the SoxR/S Promoter w/ PQ

Promoter + GFP tag

Positive  
Control

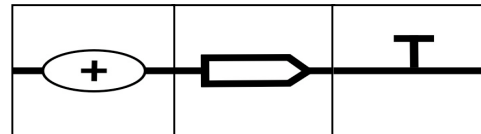
(+)



J23100

(Promoter)

+



B0034 + E0040 + B0015

(RBS)

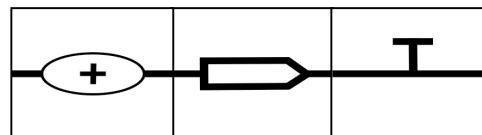
(GFP)

(TT)

GFP tag and no Promoter

Negative  
Control

(-)



B0034 + E0040 + B0015

(RBS)

(GFP)

(TT)

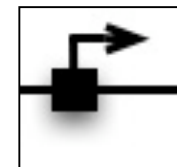
# Device 1 Controls

Testing the Blh and Raldh II

Promoter + Blh-Raldh II

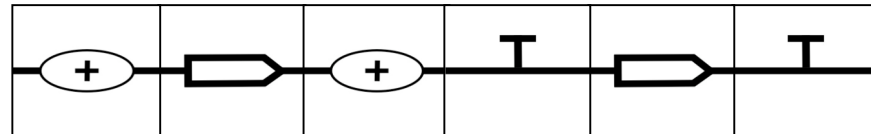
Positive  
Control

(+)



J23100  
(Promoter)

+

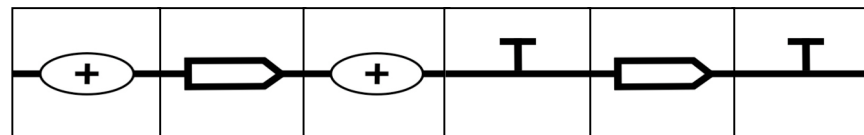


B0034 + k223035 + B0015 + B0034 + k223036 + B0015  
(RBS) (Blh gene) (TT) (RBS) (RALDH II gene) (TT)

Blh-Raldh II and no Promoter

Negative  
Control

(-)



B0034 + k223035 + B0015 + B0034 + k223036 + B0015  
(RBS) (Blh gene) (TT) (RBS) (RALDH II gene) (TT)



# Device 2

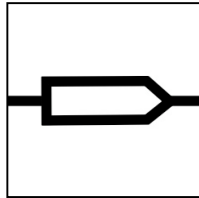
## Second Round of Cloning

2.1a hll-6-HlyAs + Terminator

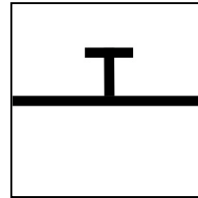
(Synthesized)

+

(#B0015)



+



k223022

(hll-6-HlyAs linker)

B0015

(TT)

Cut w/ EcoR1/Spe1

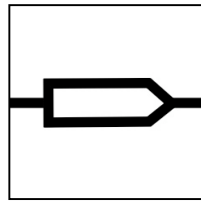
Cut w/ EcoR1/XbaI

# Device 2

## First Round of Cloning

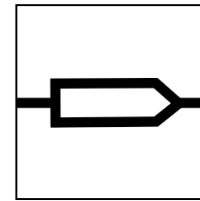
2.1b    hll-6-HlyAs    +    YFP

(will be registered)    +    (BioBricks t# I75700)



k223022  
(hll-6-HlyAs)

+



I75700  
(YFP)

Cut w/ NgoMIV/AgeI

Cut w/ NgoMIV/AgeI

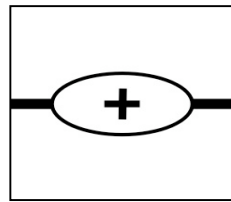


# Device 2

## First Round of Cloning

2.1c      RBS      +      TolC gene

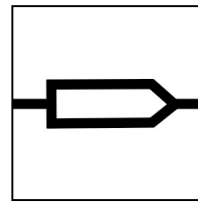
( # B0034)      +      (Synthesized part)



B0034

(RBS)

+



K223024

(TolC gene)

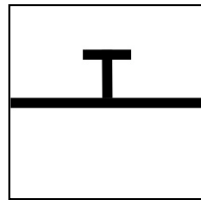
Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

# Device 2

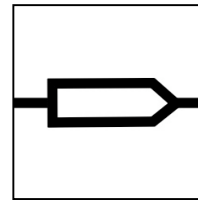
## Second Round of Cloning

2.1d Terminator + HlyR  
(#B0015) + (Synthesized)



B0015  
(TT)

+



?  
(HlyR Promoter  
element)

Cut w/ EcoR1/Spe1

Cut w/ EcoR1/XbaI

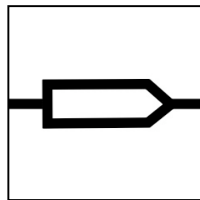
# Device 2

## First Round of Cloning

2.1e Forward Flanking Sequence (FFS) + RBS

(HlyC- Synthesized)

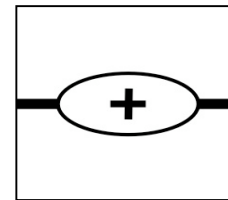
+ ( # B0034)



?

(FFS- HlyC)

+



B0034

(RBS)

Cut w/ Spe1/Pst1

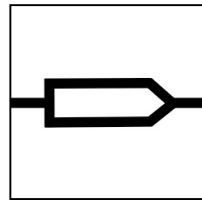
Cut w/ Xba1/Pst1

# Device 2

## Second Round of Cloning

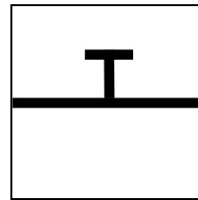
### 2.1f Rear Flanking Sequence + Terminator

(Hly B,D -Synthesized) + (#B0015)



?  
(RFS- Hly B,D)

+



B0015  
(TT)

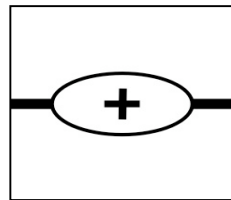
Cut w/ EcoR1/Spe1

Cut w/ EcoR1/XbaI

# Device 2

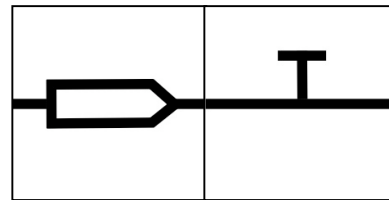
## First Round of Cloning

2.2a    RBS    +    hll-6-HlyAs-TT  
( # B0034)    +    (Cloned from 2.1a)



B0034  
(RBS)

+



K223022-B0015  
(ll-6 + HlyAs linker-TT)

Cut w/ Spe1/Pst1

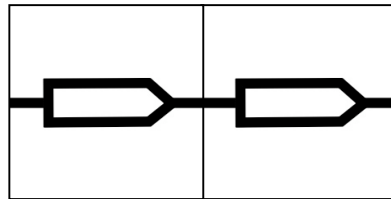
Cut w/ Xba1/Pst1

# Device 2

## First Round of Cloning

2.2b hll-6-HlyAs-YFP + Terminator

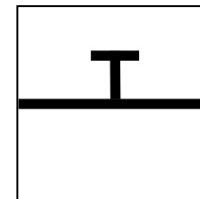
(Cloned from 2.1b) + (BioBricks # B0015)



K223022-175700

(hll-6-HlyAs-YFP)

+



B0015

(TT)

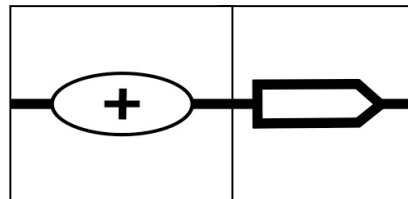
Cut w/ NgoMIV/AgeI

Cut w/ NgoMIV/AgeI

# Device 2

## First Round of Cloning

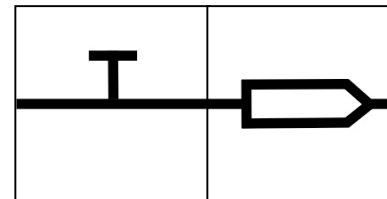
2.2c RBS-TolC gene + TT-HlyR Promoter Element  
(Cloned from 2.1c) + (Cloned from 2.1d)



B0034-K223024

(TolC gene-RBS)

+



TT-?

(TT-HlyR)

Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

# Device 2

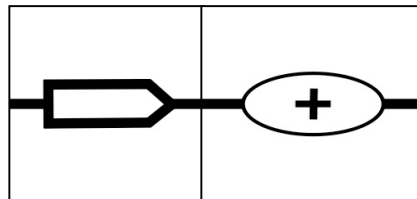
## First Round of Cloning

2.2d FFS-RBS

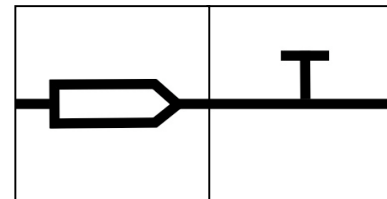
+

RFS-TT

(Cloned from step 2.1e) + (Cloned from step 2.1f)



+



?-B0034

(FFS/HlyC-RBS)

?-B0015

(RFS- Hly B,D-TT)

Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

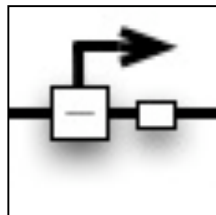


# Device 2

## First Round of Cloning

2.3a Trp Operator + RBS-hII-6-HlyAs-TT

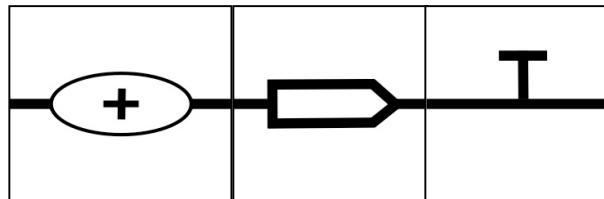
( Synthesized) + (Cloned from 2.2a)



#k223023

(Trp operator - synthesized)

+



B0034-K223022-B0015

(RBS-II-6 + HlyAs linker-TT)

Cut w/ Spe1/Pst1

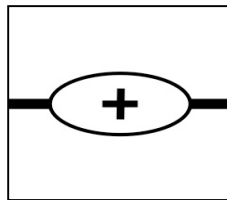
Cut w/ Xba1/Pst1

# Device 2

## First Round of Cloning

2.3b          RBS          +          hll-6-HlyAs-YFP-TT

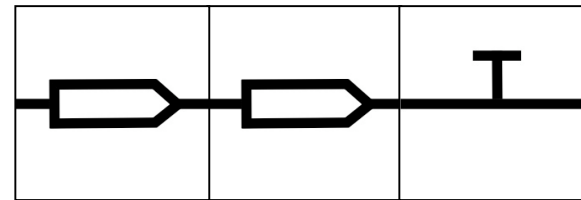
(B0034)          +          (Cloned from 2.2b)



K223022-175700

(hll-6-HlyAs-YFP)

+



B0015

(TT)

Cut w/ NgoMIV/AgeI

Cut w/ NgoMIV/AgeI

# Device 2

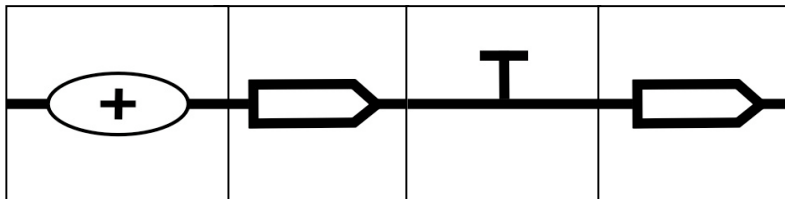
## First Round of Cloning

2.3c RBS-TolC gene-TT-HlyR +

(Cloned from 2.2c) +

FFS-RBS-RFS-TT

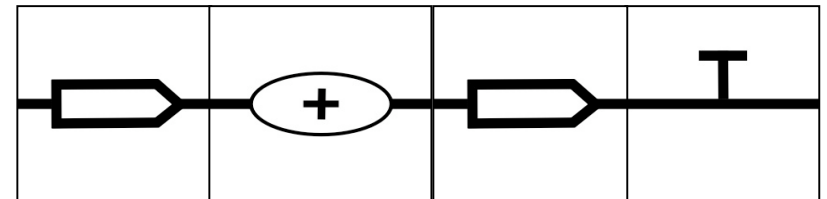
(Cloned from 2.2d)



B0034-K223024-TT-?

(TolC gene-RBS-TT-HlyR)

+



?-B0034-?-B0015

(FFS/HlyC-RBS- RFS- Hly B,D-TT)

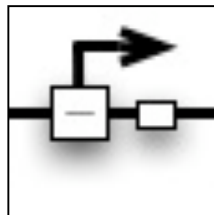
Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

# Device 2

## First Round of Cloning

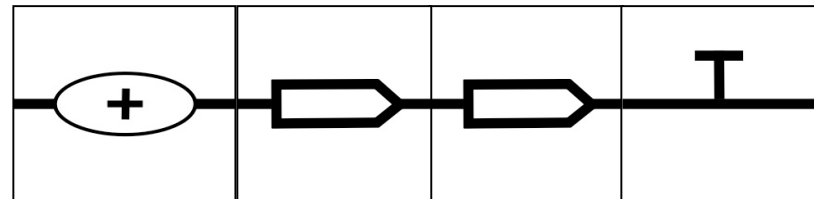
2.4a Trp Operator + RBS-hII-6-HlyAs-YFP-TT  
(Synthesized) + (Cloned from 2.3b)



#k223023

(Trp operator - synthesized)

+



B0034-K223022-175700-B0015

(RBS-hII-6-HlyAs-YFP-TT)

Cut w/ NgoMIV/AgeI

Cut w/ NgoMIV/AgeI

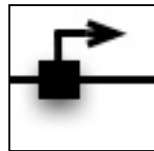
# Device 2

## First Round of Cloning

2.4b Constitutive Promoter + RBS-TolC gene-TT-HlyR-FFS-RBS-RFS-TT

(BioBrick #J23100) +

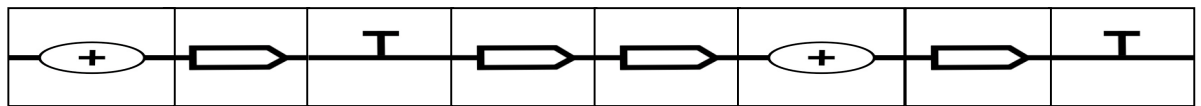
(Cloned from step 2.3c)



J23100

(Promoter)

+



B0034-K223024-TT-?-?-B0034-?-B0015

(TolC gene-RBS-TT-HlyR- FFS/HlyC-RBS- RFS- Hly B,D-TT)

Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

